

=> d his

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(FILE 'HOME' ENTERED AT 13:34:40 ON 25 MAY 2001)

FILE 'HCAPLUS' ENTERED AT 13:36:07 ON 25 MAY 2001
      E YAMAMOTO TAKUO/AU 25
L1      2 S (E3) AND (TREHALOSE)

FILE 'REGISTRY' ENTERED AT 13:39:22 ON 25 MAY 2001
L2      1 S TREHALOSE/CN

FILE 'REGISTRY' ENTERED AT 14:30:33 ON 25 MAY 2001
L3      0 S 37205-59-7/CN
L4      1 S 37205-59-7/RN

FILE 'HCAPLUS' ENTERED AT 14:32:14 ON 25 MAY 2001
      E ARTHROBACTER/CT
      E E3+ALL
      E NON REDUCING SACCHARIDE/CT
      E NON-REDUCING SACCHARIDE/CT
      E SACCHARIDE/CT
      E E4+ALL

FILE 'HCAPLUS' ENTERED AT 14:38:39 ON 25 MAY 2001

FILE 'REGISTRY' ENTERED AT 14:39:07 ON 25 MAY 2001
      SET SMARTSELECT ON
L5      SEL L2 1- CHEM :      14 TERMS
      SET SMARTSELECT OFF

FILE 'HCAPLUS' ENTERED AT 14:39:08 ON 25 MAY 2001
L6      7355 S L5

FILE 'REGISTRY' ENTERED AT 14:39:15 ON 25 MAY 2001
      SET SMARTSELECT ON
L7      SEL L4 1- CHEM :      3 TERMS
      SET SMARTSELECT OFF

FILE 'HCAPLUS' ENTERED AT 14:39:16 ON 25 MAY 2001
L8      60 S L7
L9      23 S ANTHROBACTER OR AGROBACTERIUM PSEUDOTSUGAE
L10     0 S L9 AND L6 AND L8
L11     0 S L9 AND L8
L12     0 S L8 AND ANTHROBACTER
L13     42 S L8 AND PD<19980911
L14     42 S L13 AND L6
L15     151214 S L14 AND (NON (W) REDUC) AND SACCHARIDE# OR CARBOHYDRATE#
L16     0 S L14 AND (NON (W) REDUC) AND (SACCHARIDE# OR CARBOHYDRATE#)
L17     4 S L14 AND (SACCHARIDE# OR CARBOHYDRATE#)
L18     26 S L13 AND PREP/RL
L19     0 S L13 AND PH AND DALTON#
L20     16 S L13 AND PH
L21     3 S L20 AND PI
L22     0 S L13 AND (PH OR PI) AND DALTON#
L23     0 S L13 AND DALTON#
L24     11 S L13 AND WEIGHT
L25     9 S L24 AND (PH OR PI)
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L25 ANSWER 1 OF 9 HCAPLUS COPYRIGHT 2001 ACS

ACCESSION NUMBER: 1998:676139 HCAPLUS

DOCUMENT NUMBER: 130:11851

TITLE: Purification and characterization of **trehalose phosphorylase** from the commercial mushroom *Agaricus bisporus*

AUTHOR(S): Wannet, Wim J. B.; Huub, J. M.; Den Camp, Op; Wisselink, Hendrik W.; Van Der Drift, Chris; Van Griensven, Leo J. L. D.; Vogels, Godfried D.

CORPORATE SOURCE: Department of Microbiology, Faculty of Science, University of Nijmegen, Nijmegen, NL-6525, Neth.

SOURCE: Biochim. Biophys. Acta (1998), 1425(1), 177-188

CODEN: BBACAQ; ISSN: 0006-3002

PUBLISHER: Elsevier Science B.V.

DOCUMENT TYPE: Journal

LANGUAGE: English

AB **Trehalose phosphorylase** (EC 2.4.1.64) (I) from *A.*

bisporus was purified for the 1st time from a fungus. I appears to play

a

key role in trehalose metab. in *A. bisporus* since no trehalase or trehalose synthase activities could be detected in this fungus. I catalyzes the reversible reaction of degrdn. (phosphorolysis) and synthesis of trehalose. Native I was found to have a mol. wt. of 240 kDa and to consist of 4 identical 61-kDa subunits. The **pI** of I was 4.8. The optimum temp. for both enzyme reactions was

30.degree..

The optimum **pH** ranges for trehalose degrdn. and synthesis were 6.0-7.5 and 6.0-7.0, resp. Trehalose degrdn. was inhibited by ATP and trehalose analogs, whereas the synthetic activity was inhibited by inorg. phosphate (**Pi**; $K_i = 2.0$ mM). I was highly specific for trehalose, **Pi**, glucose, and .alpha.-glucose 1-phosphate. The stoichiometry of the reaction between trehalose, **Pi**, glucose, and .alpha.-glucose 1-phosphate was 1:1:1:1 (molar ratio). The K_m values were 61, 4.7, 24 and 6.3 mM for trehalose, **Pi**, glucose, and .alpha.-glucose 1-phosphate, resp. Under physiol. conditions, *A.*

bisporus

I probably performs both synthesis and degrdn. of trehalose.

REFERENCE COUNT: 48

REFERENCE(S): (2) Baars, J; Microbiology 1994, V140, P1161 HCAPLUS
(3) Bartlett, G; J Biol Chem 1959, V234, P466 HCAPLUS
(4) Becker, A; Experientia 1996, V52, P433 HCAPLUS
(5) Belocopitow, E; Biochim Biophys Acta 1970, V198, P151 HCAPLUS
(6) Bergmeyer, H; Methoden der Enzymatischen Analyse 1974, P1250 HCAPLUS

ALL CITATIONS AVAILABLE IN THE RE FORMAT

TI Purification and characterization of **trehalose phosphorylase** from the commercial mushroom *Agaricus bisporus*

SO Biochim. Biophys. Acta (1998), 1425(1), 177-188
CODEN: BBACAQ; ISSN: 0006-3002

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key role in trehalose metab. in *A. bisporus* since no trehalase or trehalose synthase activities could be detected in this fungus. I catalyzes the reversible reaction of degrdn. (phosphorolysis) and synthesis of trehalose. Native I was found to have a mol. wt.

of 240 kDa and to consist of 4 identical 61-kDa subunits. The **pI** of I was 4.8. The optimum temp. for both enzyme reactions was 30.degree..

The optimum **pH** ranges for trehalose degrdn. and synthesis were 6.0-7.5 and 6.0-7.0, resp. Trehalose degrdn. was inhibited by ATP and trehalose analogs, whereas the synthetic activity was inhibited by inorg. phosphate (**Pi**; $K_i = 2.0$ mM). I was highly specific for trehalose, **Pi**, glucose, and .alpha.-glucose 1-phosphate. The stoichiometry of the reaction between trehalose, **Pi**, glucose, and .alpha.-glucose 1-phosphate was 1:1:1:1 (molar ratio). The K_m values were 61, 4.7, 24 and 6.3 mM for trehalose, **Pi**, glucose, and .alpha.-glucose 1-phosphate, resp. Under physiol. conditions, A.

bisporus

I probably performs both synthesis and degrdn. of trehalose.

ST **trehalose phosphorylase** mushroom; Agaricus

trehalose phosphorylase

IT Michaelis constant
(of **trehalose phosphorylase** from the com. mushroom Agaricus bisporus)

IT Agaricus bisporus
(purifn. and characterization of **trehalose phosphorylase** from the com. mushroom Agaricus bisporus)

IT **37205-59-7P, Trehalose phosphorylase**
RL: BAC (Biological activity or effector, except adverse); PRP (Properties); PUR (Purification or recovery); BIOL (Biological study); PREP (Preparation)
(purifn. and characterization of **trehalose phosphorylase** from the com. mushroom Agaricus bisporus)

L25 ANSWER 2 OF 9 HCAPLUS COPYRIGHT 2001 ACS

ACCESSION NUMBER: 1998:314518 HCAPLUS

DOCUMENT NUMBER: 129:25076

TITLE: A thermostable **trehalose phosphorylase** of Thermoanaerobium and its uses in the preparation of glucosides

INVENTOR(S): Nakada, Tetsuya; Kubota, Michio; Chaen, Hiroto; Miyake, Toshio

PATENT ASSIGNEE(S): Kabushiki Kaisha Hayashibara Seibutsu Kagaku Kenkyujo,

SOURCE: Japan
Eur. Pat. Appl., 39 pp.
CODEN: EPXXDW

DOCUMENT TYPE: Patent

LANGUAGE: English

FAMILY ACC. NUM. COUNT: 1

PATENT INFORMATION:

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
EP 841397	A2	19980513	EP 1997-308980	19971107 <--
EP 841397	A3	19990721		
R: AT, BE, CH, DE, DK, ES, FR, GB, GR, IT, LI, LU, NL, SE, MC, PT, IE, FI				
JP 10304881	A2	19981117	JP 1997-319139	19971106
US 5843748	A	19981201	US 1997-966389	19971107
US 5910436	A	19990608	US 1998-102644	19980623
US 5876975	A	19990302	US 1998-103509	19980624
US 5993889	A	19991130	US 1998-218032	19981222
PRIORITY APPLN. INFO.:			JP 1996-311232	19961108
			JP 1997-61716	19970303

US 1997-966389 19971107
US 1998-103509 19980624

AB A thermostable **trehalose phosphorylase** is obtained from microorganisms of the genus *Thermoanaerobium* that hydrolyzes trehalose in the presence of an inorg. phosphoric acid to form D-glucose and .beta.-D-glucose-1-phosphate is described. The enzyme can use .beta.-D-glucose-1-phosphate as a saccharide donor to create novel glucosides such as glucosyl-D-galactoside, that are known but rare and they can be produced on an industrial-scale and at a relatively-low cost. The enzyme has a mol. wt. of 88,000, an isoelec. point of 5.4+-.0.5, a temp. optimum of 70.degree., a pH optimum of 7.0-7.5, is activated by thiols and inhibited by divalent cations. It is stable at 60.degree. for an hour. The protein may be manufd. by expression of the cloned gene.

TI A thermostable **trehalose phosphorylase** of *Thermoanaerobium* and its uses in the preparation of glucosides

PI EP 841397 A2 19980513

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
EP 841397	A2	19980513	EP 1997-308980	19971107 <--
EP 841397	A3	19990721		

R: AT, BE, CH, DE, DK, ES, FR, GB, GR, IT, LI, LU, NL, SE, MC, PT, IE, FI

JP 10304881	A2	19981117	JP 1997-319139	19971106
US 5843748	A	19981201	US 1997-966389	19971107
US 5910436	A	19990608	US 1998-102644	19980623
US 5876975	A	19990302	US 1998-103509	19980624
US 5993889	A	19991130	US 1998-218032	19981222

AB A thermostable **trehalose phosphorylase** is obtained from microorganisms of the genus *Thermoanaerobium* that hydrolyzes trehalose in the presence of an inorg. phosphoric acid to form D-glucose and .beta.-D-glucose-1-phosphate is described. The enzyme can use .beta.-D-glucose-1-phosphate as a saccharide donor to create novel glucosides such as glucosyl-D-galactoside, that are known but rare and they can be produced on an industrial-scale and at a relatively-low cost. The enzyme has a mol. wt. of 88,000, an isoelec. point of 5.4+-.0.5, a temp. optimum of 70.degree., a pH optimum of 7.0-7.5, is activated by thiols and inhibited by divalent cations. It is stable at 60.degree. for an hour. The protein may be manufd. by expression of the cloned gene.

ST thermostable **trehalose phosphorylase** *Thermoanaerobium* gene cloning; sweetener prepn thermostable **trehalose phosphorylase** *Thermoanaerobium*; glucoside prepn thermostable **trehalose phosphorylase** *Thermoanaerobium*

IT Radish (*Raphanus sativus*)
(enzymic prepn. of glucosides for use as sweeteners for pickling of; thermostable **trehalose phosphorylase** of *Thermoanaerobium* and its uses in prepn. of glucosides)

IT Candy
Chewing gum
Chocolate
Dentifrices
Desserts
Milk preparations
Pickles
(enzymic prepn. of glucosides for use as sweeteners for; thermostable **trehalose phosphorylase** of *Thermoanaerobium* and its uses in prepn. of glucosides)

IT Sweetening agents
(enzymic prepn. of glucosides for use as; thermostable

trehalose phosphorylase of *Thermoanaerobium* and its uses in prepn. of glucosides)

IT Skin creams
(enzymic prepn. of glucosides for use in; thermostable **trehalose phosphorylase** of *Thermoanaerobium* and its uses in prepn. of glucosides)

IT Nutrients
(for intubation, enzymic prepn. of glucosides for use as sweeteners for; thermostable **trehalose phosphorylase** of *Thermoanaerobium* and its uses in prepn. of glucosides)

IT Genes (microbial)
RL: BSU (Biological study, unclassified); BUU (Biological use, unclassified); PRP (Properties); BIOL (Biological study); USES (Uses)
(for trehalose pyrophosphorylase of *Thermoanaerobium Brockii*, cloning and expression of; thermostable **trehalose phosphorylase** of *Thermoanaerobium* and its uses in prepn. of glucosides)

IT DNA sequences
(for trehalose pyrophosphorylase of *Thermoanaerobium Brockii*; thermostable **trehalose phosphorylase** of *Thermoanaerobium* and its uses in prepn. of glucosides)

IT Protein sequences
(of trehalose pyrophosphorylase of *Thermoanaerobium Brockii*; thermostable **trehalose phosphorylase** of *Thermoanaerobium* and its uses in prepn. of glucosides)

IT Jams and Jellies
(strawberry, enzymic prepn. of glucosides for use as sweeteners for; thermostable **trehalose phosphorylase** of *Thermoanaerobium* and its uses in prepn. of glucosides)

IT Milk preparations
(sweetened condensed milk, enzymic prepn. of glucosides for use as sweeteners for; thermostable **trehalose phosphorylase** of *Thermoanaerobium* and its uses in prepn. of glucosides)

IT *Thermoanaerobacter Brockii Brockii*
Thermoanaerobium
(thermostable **trehalose phosphorylase** of *Thermoanaerobium* and its uses in prepn. of glucosides)

IT Glycosides
RL: BMF (Bioindustrial manufacture); BPN (Biosynthetic preparation); BIOL (Biological study); PREP (Preparation)
(trehalose pyrophosphorylase for manuf. of; thermostable **trehalose phosphorylase** of *Thermoanaerobium* and its uses in prepn. of glucosides)

IT 208064-39-5 208064-41-9
RL: BOC (Biological occurrence); BSU (Biological study, unclassified);

CAT
(Catalyst use); PRP (Properties); BIOL (Biological study); OCCU (Occurrence); USES (Uses)
(amino acid sequence; thermostable **trehalose phosphorylase** of *Thermoanaerobium* and its uses in prepn. of glucosides)

IT 14048-34-1, .beta.-D-Glucose-1-phosphate
RL: BPR (Biological process); RCT (Reactant); BIOL (Biological study); PROC (Process)
(as substrate of trehalose pyrophosphorylase; thermostable **trehalose phosphorylase** of *Thermoanaerobium* and its uses in prepn. of glucosides)

IT 99-20-7P, Trehalose 207570-54-5P 207570-55-6P 208041-60-5P 208041-61-6P
RL: BPN (Biosynthetic preparation); BIOL (Biological study); PREP

(Preparation)
 (enzymic prepn. of; thermostable **trehalose phosphorylase** of *Thermoanaerobium* and its uses in prepn. of glucosides)

IT 50-99-7, D-Glucose, reactions 58-86-6, D-Xylose, reactions 59-23-4, D-Galactose, reactions 154-17-6, 2-Deoxyglucose 2438-80-4, L-Fucose 3416-24-8, Glucosamine 3458-28-4, D-Mannose 3615-37-0, D-Fucose 7512-17-6, N-Acetyl glucosamine
 RL: RCT (Reactant)
 (glucosidation of; thermostable **trehalose phosphorylase** of *Thermoanaerobium* and its uses in prepn. of glucosides)

IT 208064-40-8 208064-42-0
 RL: BSU (Biological study, unclassified); BUU (Biological use, unclassified); PRP (Properties); BIOL (Biological study); USES (Uses)
 (nucleotide sequence; thermostable **trehalose phosphorylase** of *Thermoanaerobium* and its uses in prepn. of glucosides)

IT 207570-56-7 207570-57-8 207570-58-9
 RL: BSU (Biological study, unclassified); PRP (Properties); BIOL (Biological study)
 (peptide of **trehalose phosphorylase** of *Thermoanaerobium*; thermostable **trehalose phosphorylase** of *Thermoanaerobium* and its uses in prepn. of glucosides)

IT **37205-59-7, Trehalose phosphorylase**
 RL: BOC (Biological occurrence); BSU (Biological study, unclassified); CAT (Catalyst use); PRP (Properties); BIOL (Biological study); OCCU (Occurrence); USES (Uses)
 (thermostable **trehalose phosphorylase** of *Thermoanaerobium* and its uses in prepn. of glucosides)

L25 ANSWER 3 OF 9 HCAPLUS COPYRIGHT 2001 ACS
 ACCESSION NUMBER: 1997:579816 HCAPLUS
 DOCUMENT NUMBER: 127:187873
 TITLE: Enzymic method for determining 1,5-anhydroglucitol for diagnosis of diabetes
 INVENTOR(S): Aisaka, Kazuo; Tazoe, Sakae; Ando, Katsuhiko; Ochiai, Keiko
 PATENT ASSIGNEE(S): Kyowa Hakko Kogyo Co., Ltd., Japan; Aisaka, Kazuo; Tazoe, Sakae; Ando, Katsuhiko; Ochiai, Keiko
 SOURCE: PCT Int. Appl., 55 pp.
 CODEN: PIXXD2
 DOCUMENT TYPE: Patent
 LANGUAGE: Japanese
 FAMILY ACC. NUM. COUNT: 1
 PATENT INFORMATION:

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
WO 9731103	A1	19970828	WO 1997-JP440	19970219 <--
W:		AU, BG, BR, CA, CN, CZ, HU, JP, KR, MX, NO, NZ, PL, RO, SG, SI, SK, UA, US, VN, AM, AZ, BY, KG, KZ, MD, RU, TJ, TM		
RW:		AT, BE, CH, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE		
CA 2218488	AA	19970828	CA 1997-2218488	19970219 <--
AU 9717327	A1	19970910	AU 1997-17327	19970219 <--
AU 722636	B2	20000810		

EP 825258 A1 19980225 EP 1997-904571 19970219 <--
R: AT, BE, CH, DE, DK, ES, FR, GB, GR, IT, LI, LU, NL, SE, MC, PT,
IE, FI
CN 1180378 A 19980429 CN 1997-190096 19970219 <--
US 6153419 A 20001128 US 1997-930709 19971016
PRIORITY APPLN. INFO.: JP 1996-32393 A 19960220
WO 1997-JP440 W 19970219

AB A method for detg. 1,5-anhydroglucitol (I) by using an enzyme that is susceptible to the inhibition by I in a concn.-dependent manner is disclosed. The method employs a compn. consisting of the enzyme (e.g. trehalase and **trehalose phosphorylase**), its substrate, and a reagent for the detn. of the enzymic reaction product. Also disclosed are a novel trehalase prepd. from Nocardia, exhibiting a pH optimum 5-6, temp. optimum 45.degree., Km <0.33 mM I or 6.7 mM trehalose, and mol. wt. 90,000 by SDS-PAGE or 400,000 by gel filtration. The method is useful for the diagnosis of diabetes. A few compn. and a blood anal. were demonstrated.

PI WO 9731103 A1 19970828

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
WO 9731103	A1	19970828	WO 1997-JP440	19970219 <--
W:		AU, BG, BR, CA, CN, CZ, HU, JP, KR, MX, NO, NZ, PL, RO, SG, SI, SK, UA, US, VN, AM, AZ, BY, KG, KZ, MD, RU, TJ, TM		
RW:		AT, BE, CH, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT,		

SE

CA 2218488	AA	19970828	CA 1997-2218488	19970219 <--
AU 9717327	A1	19970910	AU 1997-17327	19970219 <--
AU 722636	B2	20000810		
EP 825258	A1	19980225	EP 1997-904571	19970219 <--
R:		AT, BE, CH, DE, DK, ES, FR, GB, GR, IT, LI, LU, NL, SE, MC, PT, IE, FI		
CN 1180378	A	19980429	CN 1997-190096	19970219 <--
US 6153419	A	20001128	US 1997-930709	19971016

AB A method for detg. 1,5-anhydroglucitol (I) by using an enzyme that is susceptible to the inhibition by I in a concn.-dependent manner is disclosed. The method employs a compn. consisting of the enzyme (e.g. trehalase and **trehalose phosphorylase**), its substrate, and a reagent for the detn. of the enzymic reaction product. Also disclosed are a novel trehalase prepd. from Nocardia, exhibiting a pH optimum 5-6, temp. optimum 45.degree., Km <0.33 mM I or 6.7 mM trehalose, and mol. wt. 90,000 by SDS-PAGE or 400,000 by gel filtration. The method is useful for the diagnosis of diabetes. A few compn. and a blood anal. were demonstrated.

IT Catellatospora ferruginea
(trehalose phosphorylase prepd. from; enzymic method for detg. 1,5-anhydroglucitol for diagnosis of diabetes)

IT 9025-52-9, Trehalase 37205-59-7, **Trehalose phosphorylase**
RL: ARG (Analytical reagent use); BAC (Biological activity or effector, except adverse); ANST (Analytical study); BIOL (Biological study); USES (Uses)
(reagent compn. contg.; enzymic method for detg. 1,5-anhydroglucitol for diagnosis of diabetes)

L25 ANSWER 4 OF 9 HCAPLUS COPYRIGHT 2001 ACS

ACCESSION NUMBER: 1997:187051 HCAPLUS

DOCUMENT NUMBER: 126:183170

TITLE: Preparation, thermostability, and synthetic use of heat-resistant maltose phosphorylase from Bacillus

INVENTOR(S): Ishii, Keiko; Inoue, Yasushi; Tomita, Tetsuji

PATENT ASSIGNEE(S): Showa Sangyo Co., Ltd., Japan
 SOURCE: Eur. Pat. Appl., 25 pp.
 CODEN: EPXXDW
 DOCUMENT TYPE: Patent
 LANGUAGE: English
 FAMILY ACC. NUM. COUNT: 1
 PATENT INFORMATION:

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
EP 757098	A2	19970205	EP 1996-112114	19960726 <--
EP 757098	A3	19970917		
R: DE, DK, FR, GB, IT				
JP 09037780	A2	19970210	JP 1995-213005	19950731 <--
CA 2182059	AA	19970201	CA 1996-2182059	19960725 <--
US 5827715	A	19981027	US 1996-686647	19960726
US 5939308	A	19990817	US 1998-131732	19980810
PRIORITY APPLN. INFO.:			JP 1995-213005	19950731
			US 1996-686647	19960726

AB A heat-resistant maltose phosphorylase is provided from *Bacillus* sp. RK-1 and MK-1. It retains .gtoreq.80% activity after treatment in a buffer of pH 6.0 at 50-60.degree. for 15 min. Its optimum temp. was 55-70.degree., its pH optimum was 6.0-7.0 with stability retained at pH 5.5-8.0, the mol. wt. on gel filtration was 150-190 kDa and 75-90 kDa in SDS, and the isoelec. point 4.7-5.1.

The heat-resistant maltose phosphorylase differs from known maltose phosphorylases in bacterial origin, optimum temp., and thermal stability. The enzyme or bacteria contg. the enzyme can be used for prepn. of .beta.-glucose-1-phosphoric or trehalose using. By carrying out enzymic reaction at high reaction temps. using this enzyme, it is possible to prep. .beta.-glucose-1-phosphoric acid or trehalose industrially advantageously, with lowering of contamination with various germs and shortening of reaction time.

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
EP 757098 A2		19970205		
EP 757098	A2	19970205	EP 1996-112114	19960726 <--
EP 757098	A3	19970917		
R: DE, DK, FR, GB, IT				
JP 09037780	A2	19970210	JP 1995-213005	19950731 <--
CA 2182059	AA	19970201	CA 1996-2182059	19960725 <--
US 5827715	A	19981027	US 1996-686647	19960726
US 5939308	A	19990817	US 1998-131732	19980810

AB A heat-resistant maltose phosphorylase is provided from *Bacillus* sp. RK-1 and MK-1. It retains .gtoreq.80% activity after treatment in a buffer of pH 6.0 at 50-60.degree. for 15 min. Its optimum temp. was 55-70.degree., its pH optimum was 6.0-7.0 with stability retained at pH 5.5-8.0, the mol. wt. on gel filtration was 150-190 kDa and 75-90 kDa in SDS, and the isoelec. point 4.7-5.1.

The heat-resistant maltose phosphorylase differs from known maltose phosphorylases in bacterial origin, optimum temp., and thermal stability. The enzyme or bacteria contg. the enzyme can be used for prepn. of .beta.-glucose-1-phosphoric or trehalose using. By carrying out enzymic reaction at high reaction temps. using this enzyme, it is possible to prep. .beta.-glucose-1-phosphoric acid or trehalose industrially advantageously, with lowering of contamination with various germs and shortening of reaction time.

IT *Bacillus stearothermophilus*

(thermostable **trehalose phosphorylase** and; prepn.,
thermostability, and synthetic use of heat-resistant maltose
phosphorylase from Bacillus)

IT 9030-19-7P, Maltose phosphorylase 37205-59-7P, **Trehalose
phosphorylase**
RL: BAC (Biological activity or effector, except adverse); CAT (Catalyst
use); PRP (Properties); PUR (Purification or recovery); BIOL (Biological
study); PREP (Preparation); USES (Uses)
(prepn., thermostability, and synthetic use of heat-resistant maltose
phosphorylase from Bacillus)

L25 ANSWER 5 OF 9 HCAPLUS COPYRIGHT 2001 ACS

ACCESSION NUMBER: 1996:476927 HCAPLUS
DOCUMENT NUMBER: 125:108887
TITLE: Thermostable **trehalose phosphorylase**
and its preparation with Bacillus stearothermophilus
INVENTOR(S): Ishii, Keiko; Inoe, Yasushi; Tomita, Tetsuji
PATENT ASSIGNEE(S): Showa Sangyo Co, Japan
SOURCE: Jpn. Kokai Tokkyo Koho, 10 pp.
CODEN: JKXXAF
DOCUMENT TYPE: Patent
LANGUAGE: Japanese
FAMILY ACC. NUM. COUNT: 1
PATENT INFORMATION:

	PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
	JP 08131166	A2	19960528	JP 1994-295765	19941104 <--
AB	A novel trehalose phosphorylase is prepd. from the culture of Bacillus stearothermophilus strain SK-1 and characterized.				
The	enzyme exhibits a pH optimum 6.5-7.5, temp. optimum 70-75.degree., pI 4.6-5.2, and mol. wt. 110-150 kDa by gel filtration. The enzyme remains >95% active after incubating at 50-65.degree., pH 6.0 for 15 min.				
TI	Thermostable trehalose phosphorylase and its preparation with Bacillus stearothermophilus				
PI	JP 08131166	A2	19960528	Heisei	
	PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
	JP 08131166	A2	19960528	JP 1994-295765	19941104 <--
AB	A novel trehalose phosphorylase is prepd. from the culture of Bacillus stearothermophilus strain SK-1 and characterized.				
The	enzyme exhibits a pH optimum 6.5-7.5, temp. optimum 70-75.degree., pI 4.6-5.2, and mol. wt. 110-150 kDa by gel filtration. The enzyme remains >95% active after incubating at 50-65.degree., pH 6.0 for 15 min.				
ST	trehalose phosphorylase prepn Bacillus				
IT	Bacillus stearothermophilus (thermostable trehalose phosphorylase and prepn. with Bacillus stearothermophilus)				
IT	37205-59-7P, Trehalose phosphorylase RL: BPN (Biosynthetic preparation); PRP (Properties); BIOL (Biological study); PREP (Preparation) (thermostable trehalose phosphorylase and prepn. with Bacillus stearothermophilus)				

L25 ANSWER 6 OF 9 HCAPLUS COPYRIGHT 2001 ACS

ACCESSION NUMBER: 1995:906143 HCAPLUS

DOCUMENT NUMBER: 123:309235
 TITLE: Purification and characterization of **trehalose phosphorylase** from *Micrococcus varians*
 AUTHOR(S): Kizawa, Hideki; Miyagawa, Ken-ichiro; Sugiyama, Yoshio
 CORPORATE SOURCE: Integrated Technology Laboratories, Takada Chemical Industries, Ibaraki, 300-42, Japan
 SOURCE: Biosci., Biotechnol., Biochem. (1995), 59(10), 1908-12
 CODEN: BBBIEJ; ISSN: 0916-8451
 DOCUMENT TYPE: Journal
 LANGUAGE: English

AB **Trehalose phosphorylase** (EC 2.4.1.64), which catalyzes the reversible reaction of phosphorolysis and synthesis of trehalose, was purified to homogeneity from a cell-free ext. of *Micrococcus varians* strain No. 39. The enzyme was shown to have a mol. wt. of 570,000 to 580,000 by gel filtration, and to have a subunit of mol. wt. of 105,000 by SDS-PAGE. The stoichiometry of the reaction between trehalose, **Pi**, glucose, and .beta.-glucose 1-phosphate was 1:1:1:1 (molar ratio). The enzyme had high specificity for trehalose,

glucose, and .beta.-glucose 1-phosphate. The Kms for trehalose, **Pi**, glucose, and .beta.-glucose 1-phosphate were 10, 3.1, 23, and 38 mM, resp. The kcats were 200 s⁻¹ for trehalose phosphorolysis and 660 s⁻¹ for trehalose synthesis. The enzyme was inhibited by validamycin A, validoxylamine A, 1-deoxynojirimycin, and Cu²⁺ during trehalose phosphorolysis, and by Cu²⁺, Zn²⁺, and Ni²⁺ during trehalose synthesis. Inhibition competitive against trehalose was noted with validamycin A, validoxylamine A, and 1-deoxynojirimycin. Initial velocity, product inhibition, and dead-end inhibition studies suggested that both trehalose phosphorolysis and trehalose synthesis proceeded through an ordered Bi Bi mechanism.

TI Purification and characterization of **trehalose phosphorylase** from *Micrococcus varians*

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ST **trehalose phosphorylase** *Micrococcus*
 IT Kinetics, enzymic
 Michaelis constant
Micrococcus varians

(purifn. and characterization of **trehalose phosphorylase** from *Micrococcus varians*)

IT 7440-02-0, Nickel, biological studies 7440-50-8, Copper, biological studies 7440-66-6, Zinc, biological studies 19130-96-2, 1-Deoxynojirimycin 37248-47-8, Validamycin A 38665-10-0, Validoxylamine A
 RL: BAC (Biological activity or effector, except adverse); BIOL (Biological study)
 (purifn. and characterization of **trehalose phosphorylase** from *Micrococcus varians*)

IT 37205-59-7P, **Trehalose phosphorylase**
 RL: BAC (Biological activity or effector, except adverse); BPR (Biological process); PRP (Properties); PUR (Purification or recovery); BIOL (Biological study); PREP (Preparation); PROC (Process)
 (purifn. and characterization of **trehalose phosphorylase** from *Micrococcus varians*)

IT 50-99-7, D-Glucose, biological studies 99-20-7, Trehalose 14048-34-1, .beta.-Glucose 1-phosphate 14265-44-2, Phosphate, biological studies
 RL: BPR (Biological process); BIOL (Biological study); PROC (Process)
 (purifn. and characterization of **trehalose phosphorylase** from *Micrococcus varians*)

L25 ANSWER 7 OF 9 HCAPLUS COPYRIGHT 2001 ACS
 ACCESSION NUMBER: 1995:538169 HCAPLUS
 DOCUMENT NUMBER: 123:7925
 TITLE: Production and application of maltose phosphorylase and **trehalose phosphorylase** by a strain of *Plesiomonas*
 AUTHOR(S): Yoshida, Masahiro; Nakamura, Nobuyuki; Horikoshi, Koki
 CORPORATE SOURCE: Res. Inst., Nihon Shokuhin Kako Co., Ltd., Fuji, 417, Japan
 SOURCE: Oyo Toshitsu Kagaku (1995), 42(1), 19-25
 CODEN: OTKAE3; ISSN: 1340-3494
 DOCUMENT TYPE: Journal
 LANGUAGE: English

AB A strain of *Plesiomonas* capable of producing intracellular thermostable maltose phosphorylase (MP) and **trehalose phosphorylase** (TP), which were useful for the prodn. of trehalose from maltose, was isolated from mud of a Japanese seashore. The isolate (SH-35) grew well among the ranges of pH 6-9, and temp. 15-45.degree. with optima at pH 7.0 and 37.degree. by shaking cultivation, though the max. yield of these enzymes were obtained at pH 7.5 and 34.degree. for MP and pH 8.0 and 37.degree. for TP in a medium contg. maltose as a carbon source and mixt. of polypepton-S, yeast ext. and urea as nitrogen sources. Optimum pH and temp. for producing trehalose were pH 7-8 and 55-60.degree. in the presence of 10-40% (wt./wt.) maltose and 5-50 mM inorg. phosphate, and about 60% (as dry basis) of maltose was converted into trehalose by the simultaneous action of both enzymes before and after extn. from cells under the best conditions. These microbial and enzymic characteristics are consistent with the industrial prodn. of trehalose from maltose.

TI Production and application of maltose phosphorylase and **trehalose phosphorylase** by a strain of *Plesiomonas*
 SO Oyo Toshitsu Kagaku (1995), 42(1), 19-25
 CODEN: OTKAE3; ISSN: 1340-3494

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isolated from mud of a Japanese seashore. The isolate (SH-35) grew well among the ranges of pH 6-9 and temp. 15-45.degree. with optima at pH 7.0 and 37.degree. by shaking cultivation, though the max. yield of these enzymes were obtained at pH 7.5 and 34.degree. for MP and pH 8.0 and 37.degree. for TP in a medium contg. maltose as a carbon source and mixt. of polypepton-S, yeast ext. and urea as nitrogen sources. Optimum pH and temp. for producing trehalose were pH 7-8 and 55-60.degree. in the presence of 10-40% (wt./wt.) maltose and 5-50 mM inorg. phosphate, and about 60% (as dry basis) of maltose was converted into trehalose by the simultaneous action of both enzymes before and after extn. from cells under the best conditions. These microbial and enzymic characteristics are consistent with the industrial prodn. of trehalose from maltose.

- ST Plesiomonas maltose **trehalose phosphorylase** prodn application
 IT Plesiomonas
 (prod. and application of maltose phosphorylase and **trehalose phosphorylase** by strain of Plesiomonas)
 IT 99-20-7P, Trehalose
 RL: BMF (Bioindustrial manufacture); BIOL (Biological study); PREP (Preparation)
 (manuf. of, from maltose by maltose phosphorylase and **trehalose phosphorylase** from Plesiomonas)
 IT 9030-19-7P, Maltose phosphorylase **37205-59-7P, Trehalose phosphorylase**
 RL: BAC (Biological activity or effector, except adverse); BMF (Bioindustrial manufacture); BIOL (Biological study); PREP (Preparation)
 (prod. and application of maltose phosphorylase and **trehalose phosphorylase** by strain of Plesiomonas)
 IT 69-79-4, Maltose
 RL: BPR (Biological process); BSU (Biological study, unclassified); BIOL (Biological study); PROC (Process)
 (trehalose manuf. from, by maltose phosphorylase and **trehalose phosphorylase** from Plesiomonas)

L25 ANSWER 8 OF 9 HCAPLUS COPYRIGHT 2001 ACS

ACCESSION NUMBER: 1975:27655 HCAPLUS

DOCUMENT NUMBER: 82:27655

TITLE: Metabolism of trehalose in Euglena gracilis. Partial purification and some properties of

phosphoglucumutase

acting on .beta.-glucose 1-phosphate

AUTHOR(S): Belocopitow, Enrique; Marechal, Luis R.

CORPORATE SOURCE: Inst. Invest. Bioquim. "Fundacion Campomar", Buenos Aires, Argent.

SOURCE: Eur. J. Biochem. (1974), 46(3), 631-7

CODEN: EJBCAI

DOCUMENT TYPE: Journal

LANGUAGE: English

AB Phosphoglucumutase (I) for .beta.-D-glucose 1-phosphate (II) was purified 460-fold from cell-free exts. of E. gracilis var. bacillaris by treatment with protamine sulfate, gel filtration on Sephadex G-100, and chromatog. on a DEAE-cellulose column. I converted II reversibly into D-glucose 6-phosphate (III). The optimum pH of the reaction was 7.0 and the equil. const., II/III was 0.035. I required .beta.-D-glucose 1,6-diphosphate and a divalent cation such as Mg²⁺, Co²⁺, or Mn²⁺. Measurements on Sephadex G-100 gave an apparent mol. wt. of .apprx.27,000. I, together with a **trehalose phosphorylase** found in the same Euglena exts., would constitute a new catabolic pathway for trehalose.

SO Eur. J. Biochem. (1974), 46(3), 631-7
CODEN: EJBCAI
AB Phosphoglucosyltransferase (I) for .beta.-D-glucose 1-phosphate (II) was purified 460-fold from cell-free exts. of E. gracilis var. bacillaris by treatment with protamine sulfate, gel filtration on Sephadex G-100, and chromatog. on a DEAE-cellulose column. I converted II reversibly into D-glucose 6-phosphate (III). The optimum pH of the reaction was 7.0 and the equil. const., II/III was 0.035. I required .beta.-D-glucose 1,6-diphosphate and a divalent cation such as Mg²⁺, Co²⁺, or Mn²⁺. Measurements on Sephadex G-100 gave an apparent mol. wt. of .apprx.27,000. I, together with a **trehalose phosphorylase** found in the same Euglena exts., would constitute a new catabolic pathway for trehalose.

L25 ANSWER 9 OF 9 HCAPLUS COPYRIGHT 2001 ACS

ACCESSION NUMBER: 1972:402254 HCAPLUS

DOCUMENT NUMBER: 77:2254

TITLE: Metabolism of trehalose in Euglena gracilis. I. Partial purification and some properties of **trehalose phosphorylase**

AUTHOR(S): Marechal, Luis R.; Belocopitow, Enrique

CORPORATE SOURCE: Inst. Invest. Bioquim. Fund. Campomar, Buenos Aires, Argent.

SOURCE: J. Biol. Chem. (1972), 247(10), 3223-8

CODEN: JBCHA3

DOCUMENT TYPE: Journal

LANGUAGE: English

AB **Trehalose phosphorylase**, an enzyme found in cell-free exts. of E. gracilis var. bacillaris, was purified 75-fold by treatment with protamine sulfate, centrifugation at 200,000 g, and chromatog. in a column of hydroxylapatite. This enzyme catalyzes the reversible phosphorolytic splitting of trehalose, yielding .beta.-glucose 1-phosphate

and glucose as products. The optimum pH of the reaction was 7.0 for phosphorolysis and 6.3 for the synthesis of trehalose. The equil. const. changes with pH. It was 4.2 at pH 7.0 and 17 at pH 6.3. The enzyme is very unstable in the absence of inorg. phosphate, .alpha.- or .beta.-glucose 1-phosphate. Measurements in sucrose gradient gave a mol. wt. of 344,000. This enzyme together with a phosphoglucosyltransferase for .beta.-glucose 1-phosphate found

in the same Euglena exts. would constitute a new catabolic pathway for trehalose.

TI Metabolism of trehalose in Euglena gracilis. I. Partial purification and some properties of **trehalose phosphorylase**

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in together with a phosphoglucomutase for .beta.-glucose 1-phosphate found
the same Euglena exts. would constitute a new catabolic pathway for
trehalose.

ST **trehalose phosphorylase** Euglena

IT Euglena gracilis

(**trehalose phosphorylase** of)

IT **37205-59-7**

RL: BOC (Biological occurrence); BIOL (Biological study); OCCU
(Occurrence)

(of Euglena gracilis)